

SOME ASPECTS OF NATURAL-ABUNDANCE NITROGEN-15 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

by John D. Roberts

ABSTRACT

Some of the problems involved in obtaining useful nuclear magnetic resonance signals from nitrogen-15 at the natural-abundance level in organic compounds are discussed. Special attention is given to the structural, stereochemical, and solvent effects on the ^{15}N NMR spectra of amines. Applications of ^{15}N NMR spectra to structural analysis of sulfonamides, oximes, imines, and peptides are presented along with procedures for determining the exchange rates of N-H protons through acid or base catalysis.

Nuclear magnetic resonance (NMR) spectroscopy has expanded like the cosmic "big bang" during the thirty years following the initial developments by Bloch¹ and Purcell,² and the end seems nowhere in sight. No other form of spectroscopy has so many applications or provides such deep insight into structural problems of substances as liquids or in solutions. Other than quantum mechanics, there has been no greater gift of modern physics to chemistry.

The expansion of NMR in chemistry has hardly been uniform, because the technology for many possible applications has developed unevenly. As each new breakthrough has come about, however, exploitation of it has usually been very rapid indeed. Cases in point are ^{13}C and ^{15}N NMR spectroscopy at the low natural-abundance levels of these isotopes. The pioneering work of Lauterbur³ on ^{13}C NMR (1957-1961) and that of Grant⁴ starting in 1964 clearly established the potential of ^{13}C NMR for structural analysis, but the difficulty in taking high-resolution spectra at the natural-

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abundance level, where the sensitivity is 1.76×10^{-3} that of ^1H , was not overcome until a series of technological improvements culminated in 1966–1968. These included 1) greater magnetic field homogeneity, which allowed use of larger samples; 2) more stable magnetic fields and RF oscillators, which allowed time averaging of weak signals; and 3) broadband proton decoupling, which greatly increased the signal strength through collapsing the C-H spin-spin splittings and generation of a very favorable nuclear Overhauser enhancement. Subsequent application of the pulsed Fourier transform (FT) NMR technique supplied the final impetus for conversion of natural-abundance ^{13}C NMR into an essentially routine (albeit expensive) procedure for structure analysis that is now in very widespread use.^{5,6,7}

Nitrogen NMR has a little different history. The chemical shift was in fact discovered by Proctor and Yu⁸ for ammonium nitrate and much effort has been expended on developing ^{14}N NMR as a structural tool, principally by Klein, Richards, Witanowski, and their coworkers.⁹ Although the range of nitrogen NMR chemical shifts is very large, however, the substantial quadrupole moment of the ^{14}N nucleus in conjunction with substantial electrical field gradients at nitrogen nuclei usually causes rapid relaxation and very substantial line broadening.¹⁰ These line broadenings may be as large as 200 ppm and hence may completely obscure the fine details of the spectrum, especially spin-spin couplings, which with ^{14}N are usually less than 100 Hz. Conspicuous exceptions are provided by isocyanides and compounds that have a high degree of electrical symmetry around nitrogen, but these are not of general enough occurrence to allow ^{14}N NMR to be classed as a high-resolution method, at least in comparison with the NMR of spin 1/2 nuclei.

The ^{15}N nucleus offers a different problem. It does have spin 1/2, so that there is no quadrupole-induced relaxation, but it has a small magnetic moment (1/10 that of the proton), and a low natural abundance of 0.365%. Thus, at the natural-abundance level at constant field, the sensitivity of detection of ^{15}N relative to ^1H is down by a factor of 3×10^{-6} . Furthermore, because the gain in signal-to-noise ratio from time-averaging of signals improves only as the square root of the time of observation, then, on the critical time basis, ^{15}N is worse than ^1H NMR by a factor of about 10^{-11} ! Isotopic enrichment of ^{15}N to the 90% level improves this by a factor of 10^5 , and the first important surveys of the possibilities of ^{15}N for structural analysis used enriched materials.¹¹ Nonetheless, although ^{15}N -enriched samples give excellent spectra,¹² the history of ^{13}C NMR shows that rapid and general progress simply does not occur until spectra can be taken routinely at the natural-abundance level. One of the first natural-abundance spectra of ^{15}N was of 85% hydrazine taken with time averaging using a 10-mm sample tube without proton decoupling and continuous-wave excita-

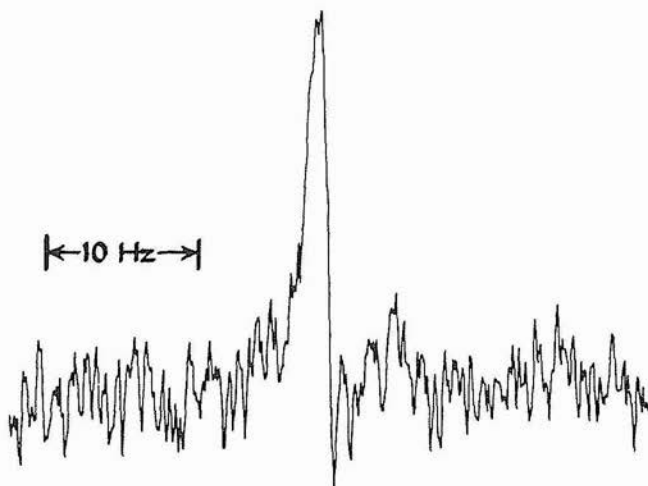


FIG. 1. TIME-AVERAGED, continuous-wave, natural-abundance ^{15}N spectrum of 85% diazane at 6.07 MHz, contained in a 10-mm sample tube, taken with 303 scans at 50 sec per scan.¹³

tion at 6.07 MHz (figure 1).¹³ This is essentially an optimum sample through being about 40 M in nitrogen, but it still required a few hours to give an acceptable signal-to-noise ratio.

Further progress became possible through proton decoupling for samples with favorable Nuclear Overhauser Enhancement (NOE), which usually means having nitrogens with attached hydrogens and reasonably short T_1 relaxation times. The NOE for nitrogen is different from that of carbon in that it results in an emission rather than an absorption signal.¹⁴ Thus, depending on the efficiency of the NOE, a ^{15}N resonance may be upright, reduced to insignificance by having absorption just cancel emission, or strongly emissive (maximum = -2.93 times the signal strength of the unsplit absorption signal). The T_1 relaxation times of ^{15}N are highly variable, being greatly influenced by structure and by traces of paramagnetic impurities (especially for water solutions of compounds with nitrogens that carry unshared electron pairs).

Many T_1 's for ^{15}N , even those with directly bonded hydrogens, such as simple amines, are in the range from 30-100 seconds, while those of tertiary amines may be on the order of 10^3 seconds. Such relaxation times make taking spectra by the FT technique rather inefficient. The use of paramagnetic relaxing agents is often recommended to shorten the T_1 values, but this is not always helpful because 1) any favorable NOE is cancelled, 2) the linewidths are often increased so much that the signal/noise ratios are not much improved, and 3) there can be significant changes in chemical

shift as the result of contact- or pseudocontact-shift contributions resulting from interaction of the paramagnetic species with the nitrogens whose relaxation times are desired to be shortened. Fortunately, the efficiency of dipolar relaxation of ^{15}N by directly attached or neighboring protons increases substantially on slowing molecular reorientations so that as the correlation time, τ_c , becomes greater (at least to about 10^{-8} seconds), the efficiency of taking FT spectra greatly improves. This is especially important for large, biochemically interesting molecules.¹⁵

The technology that moved natural-abundance ^{15}N NMR into the arena of more routine instrumental techniques was development of relatively large-bore superconducting magnets with a high degree of homogeneity, ~ 5 parts in 10^9 over a substantial volume, thus permitting more nuclei to be placed within the active volume of the probe—in essence, the “milk-bottle” approach, the feasibility of which was demonstrated at lower magnetic fields for ^{13}C by Allerhand and coworkers.¹⁶ The one serious difficulty with this approach is the very large sample sizes required. The WH-180 spectrometer developed for our research at the California Institute of Technology by Bruker Magnetics works very well with 25-mm sample tubes and, with that size of tube, requires a minimum of 18 ml of sample. Use of 30-mm tubes, with about 40 ml of sample, gave no advantage for routine spectra because of reduced field homogeneity.

One might well argue that there is nothing “routine” about a technique requiring 20 ml of sample, but this is surely a value judgment because it depends so much on the nature of the material, especially on the ^{15}N relaxation times, and also on the length of time the spectroscopist is willing to devote to taking spectra. Thus, as regards time, if the ratio of signal-to-noise for a given set of resonances is required to be improved by a factor of 5 after 2 hours of taking a spectrum, the sample has to be an important one to devote 50 hours to achieve this objective. As mentioned earlier, the relaxation-time problem can be acute for small molecules and even neat *N*-methylazacyclopentane (~ 10 M) gives only a very weak signal when pulsed with 1-2 minute repetition rates.¹⁷ Contrariwise, 0.08 M cyanocobalamine, a very much larger molecule whose amide nitrogen nuclei relax with greater efficiency, because of a greater τ_c , can be pulsed with a repetition rate of 0.82 second, and gives an excellent spectrum of these nitrogens after 18 hours (figure 2). A still greater effect is observed for the enzyme lysozyme, which at 0.009 M with a 0.82 sec repetition rate was found to give a useful spectrum in 21 hours. For this substance at the given concentration, the relaxation times may be close to the optimum values for rapid pulsing, without serious line broadening. Nonetheless these same relaxation times may be sufficiently short as to quench the NOE's of some of the peptide nitrogens (note the upward resonances of figure 3).

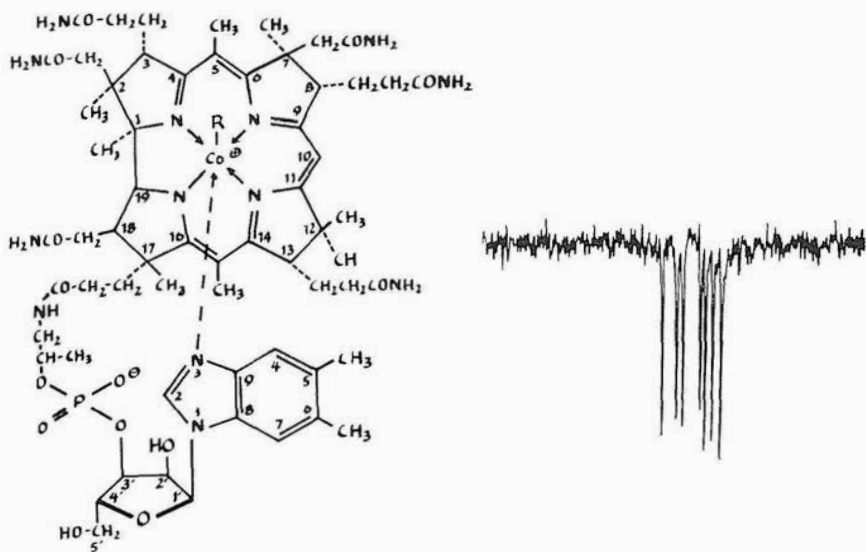


FIG. 2. PULSED FOURIER-TRANSFORM NMR spectrum of cyanocobalamin (vitamin B₁₂) as an 0.08 M solution in 1:1 ethanol-water at 18.25 MHz. The spectrum was taken with 3 mW of proton decoupling, 78,811 pulses (90° flip angle), and a repetition rate of 0.82 sec. The only peaks observed are those that can be attributed to the seven amide ($-\text{CONH}_2$) groups.¹⁵

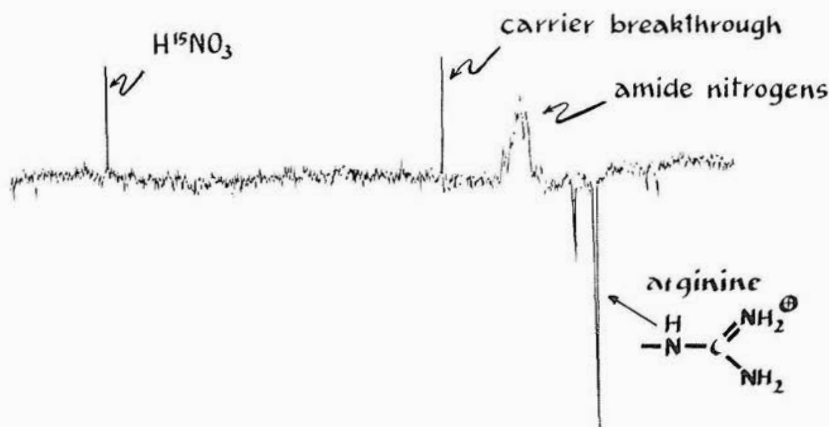


FIG. 3. NATURAL-ABUNDANCE ^{15}N NMR spectrum of 0.009 M egg-white lysozyme in water at pH 3.9. A total of 93,287 transients was taken, with the other spectral parameters being the same as in figure 2.¹⁵

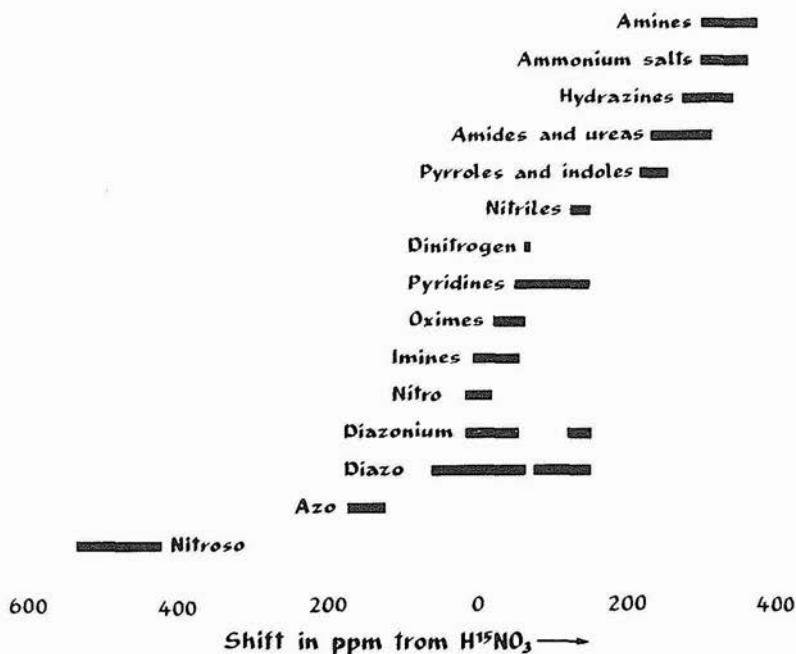


FIG. 4. SOME RANGES OBSERVED for nitrogen chemical shifts as a function of structure.

When the principal obstacle to widespread use of ^{15}N was overcome and literally thousands of substances could be studied, an essentially explosive development period ensued to which many investigators have contributed—most notably Randall (Queen Mary College), Lichter (Hunter College), Levy (Florida State University), and their coworkers. No attempt will be made here to provide a comprehensive review of the current status of ^{15}N NMR. Instead, only a few topics will be covered, largely drawn from research on steric, hydrogen-bonding, protonation, and complexation effects on ^{15}N chemical shifts and the relation between ^{15}N and ^{13}C shifts in analogously constituted compounds.

Structural elucidation assisted by ^{15}N NMR depends very largely on chemical-shift differences. Most spin-spin couplings involving ^{15}N , except for those arising from directly bonded (and not rapidly exchanging) protons, are only a few Hz because of the small magnetic moment of ^{15}N . Thus they usually have limited utility for structural analysis. Chemical shifts of nitrogen span a very considerable range (figure 4), however, as is typical of magnetic nuclei of atoms such as C, F, P, and so on (but not H or Li) that use p orbitals in bond formation. The importance of p orbitals is that, under the influence of a magnetic field, electrons in these orbitals undergo a forced circulation that can generate a very sizable paramagnetic

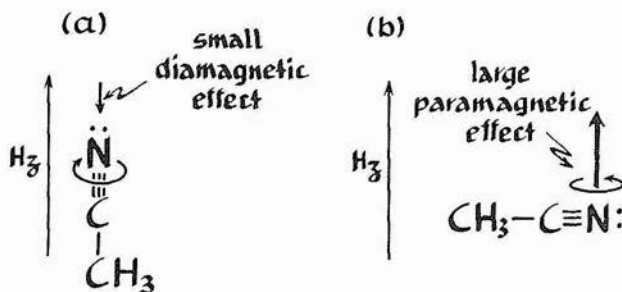


FIG. 5. DIAMAGNETIC AND PARAMAGNETIC ELECTRON CIRCULATIONS postulated for ethanenitrile as a function of orientation in a magnetic field H_z .

effect ("second-order paramagnetic effect") directly at the nucleus undergoing absorption. Because the bonding orbitals normally have a full complement of electrons, circulation of electrons in these filled orbitals produces diamagnetic shielding. To account for the paramagnetic circulation, it is assumed that excited-state wave functions contribute sufficiently to the ground-state electronic configuration to give the observed paramagnetic influences. In this context, the idea that an "electron-promotion" energy should be important to the shifts is a very natural one.

It should be recognized that these paramagnetic circulations do not correspond to the diamagnetic circulations that are used to account for the shifts of the protons of benzene or ethyne. Thus, for a triple bond such as in ethanenitrile, when the molecule is *parallel* to the magnetic field, diamagnetic circulation around the bond produces a diamagnetic effect along the bond axis (figure 5a). However, as has been shown by Pople,¹⁸ this diamagnetic effect is too small to account for the shift of ethyne protons and must also be too small to have much influence on the shift of ^{15}N in ethanenitrile. The second-order paramagnetic effect operates most effectively when the long axis of the ethanenitrile molecule is *perpendicular* to the magnetic field, and at least some (and probably a lot) of the circulation appears to involve one of the electrons of the unshared electron pair on nitrogen and a promotion energy that reasonably corresponds to the $n \rightarrow \pi^*$ excitation energy (figure 5b). The second-order paramagnetic effect is in agreement with the fact that the ^{15}N shift of $\text{CH}_3\text{C}\equiv\text{N}$ is about 235 ppm *downfield* from the shift of $(\text{CH}_3)_3\text{N}$: for which there is no low-lying π^* excited state. Furthermore, when $\text{CH}_3\text{C}\equiv\text{N}$ is converted by strong acid to the corresponding conjugate acid, $\text{CH}_3\text{C}\equiv\text{NH}^+$, in which the nitrogen resonance is expected by simple theory to be shifted *downfield* because of its positive charge, the opposite occurs and the nitrogen resonance shifts *upfield* by more than 100 ppm.¹⁹ This shift change is very reasonable on the basis of

the second-order paramagnetic effect because the $n \rightarrow \pi^*$ transition in $\text{CH}_3\text{C}\equiv\text{N}$: becomes a $\sigma \rightarrow \pi^*$ transition in $\text{CH}_3\text{C}\equiv\overset{\oplus}{\text{N}}-\text{H}$, which should have a much greater energy, thus greatly reducing mixing into the ground state of excited-state electronic configurations that correspond to $n \rightarrow \pi^*$ (or similar) transitions. Further confirmation of the general correctness of these formulations comes from the fact that protonation of $(\text{CH}_3)_3\text{N}$: results in a *downfield* shift of 13 ppm.²⁰

The message of the discussion above is that a nitrogen with an unshared pair that is part of a π -electron system can be expected to have a substantial downfield shift compared to that of a saturated nitrogen. Also, if the unshared pair is protonated or otherwise perturbed so as to increase the promotion energy, then there should be a sizable upfield shift. We will see how this works out in practice.

Before proceeding further, I should mention the shift scales and reference compounds for ^{15}N chemical shifts, because there is no uniformity about this at present. One problem is that, as yet, no one has come up with a suitably inert, easily labeled, widely soluble nitrogen analog of tetramethylsilane to use as an internal shift standard. Consequently, external standards are almost universally used, and in our laboratory we have found it very convenient to use a 1 M solution of ^{15}N -labeled nitric acid in deuterium oxide as an external standard, contained in a 5-mm tube centered in our 20-mm sample tubes. This mixture has the advantage of having deuterium nuclei available to provide a field-frequency lock signal. It is disadvantageous because the position of the reference should be corrected for temperature. Nitromethane labeled with ^{15}N and substituted with deuterium (CD_3NO_2) is a less temperature-sensitive reference, but one that is more difficult and expensive to prepare. The resonance of our nitric-acid standard is 6.2 ppm upfield from neat nitromethane.

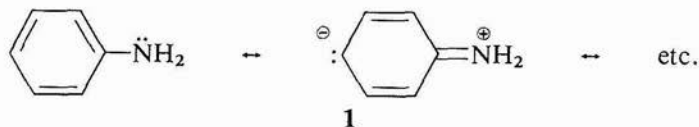
Another unresolved problem with using the resonances of either nitromethane or nitric acid as a reference is that the vast majority of ^{15}N shifts are then upfield. Initially, NMR spectroscopists liked downfield standards, the epitome of which would be the bare gaseous nuclei. For proton spectra, water and benzene were once widely used; for ^{13}C spectra, carbon disulfide was the reference of choice, and so on. However, the advent of tetramethylsilane as a reference gave a convenient upfield standard for both proton and carbon spectra, and finally some misguided committees decided that *all* NMR shifts should be reported in exactly the same way. Adherence to this dictum means either that all shifts upfield of a standard of reference must be given *negative signs* (a potent device for promoting misunderstanding and mistakes), or else that everything must be referenced to an upfield standard, however inconvenient on other grounds. The present situation is far from desirable, but the best solution is more likely to be achieved by

further research rather than by rules set up by committees or journal editors unrepresentative of the workers in the field.

We will be mostly interested in this paper in ^{15}N shift *differences*, but where shifts are given numerical values, these will be in ppm *upfield* from our external nitric-acid standard. To convert to ppm *upfield* from nitromethane, add 6.2 ppm. For ppm *downfield* from 2 M urea in water, 2 M tetramethylammonium chloride in water, and 2 M ammonium nitrate (ammonium resonance) in water, use the relationship $-(\delta_{\text{H}^{15}\text{NO}_3} - \delta_{\text{STND}})$, where δ_{STND} for each of the substances above is 298.7, 332.8, and 355.0 ppm, respectively.²¹

In 1967, Spielvogel and Purser²² made the very fruitful discovery that there is a correlation between ^{11}B NMR shifts and ^{13}C shifts in reasonably corresponding compounds. Applied to ^{15}N shifts, what this means is that one might expect the ^{13}C shift of C1 of butane to correlate with the nitrogen shift of propanamine, the ^{13}C shift of C2 of 2-methylbutane with the nitrogen shift of *N,N*-dimethylethanamine and so on. This possibility has been carefully investigated and found to have great merit for a variety of primary and secondary amines (figure 6).²³ The dependence is linear to a very high degree over about 90 ppm, but, for reasons not yet fully understood, primary and secondary amines are found to fall along lines separated by about 10 ppm, at ^{15}N shifts near 330 ppm, from nitric acid with somewhat different slopes. The slopes of the correlation lines show the ^{15}N shifts are about twice as sensitive to variations in structure as are ^{13}C shifts.

An important point about the amine shifts correlated in figure 6 is that, in all save one of the amines, the nitrogens are not part of a π -electron system. The exception, benzenamine (compound 1), is expected to be different because of possible conjugation of the unshared pair of electrons on nitrogen with the benzene ring. This sort of conjugation is not expected for the methyl carbon of methylbenzene, and thus it is not surprising that



the ^{15}N shift of benzenamine is about 18 ppm farther downfield than expected from the correlation line. We can also understand why conversion of compound 1 to benzenammonium ion causes an upfield shift of its ^{15}N resonance.¹¹

At the time the data for figure 6 were being collected, it was not possible to obtain useful ^{15}N spectra of tertiary amines, and it could not be determined whether such amines would give a third correlation line in a plot such as figure 6. Later research with a substantial number of substituted

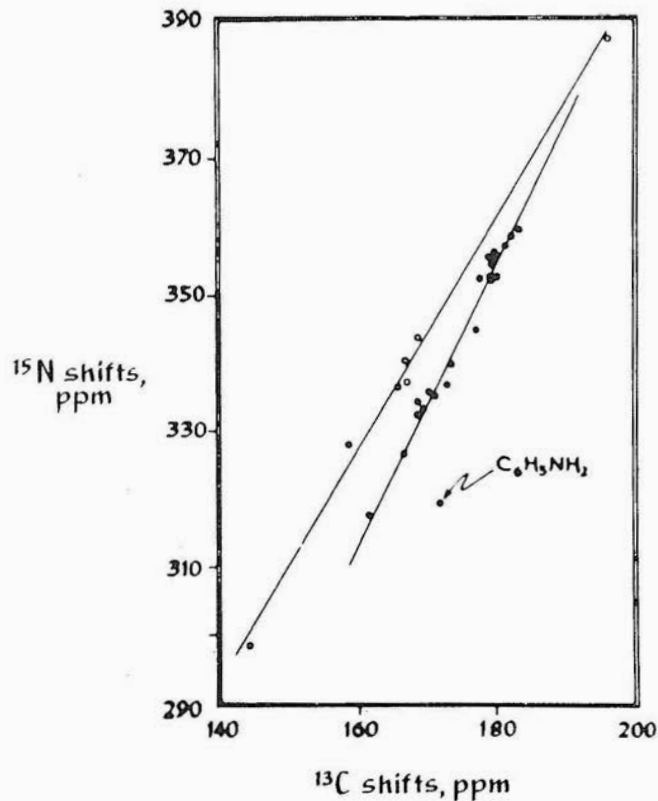


FIG. 6. CORRELATIONS OF ^{15}N CHEMICAL SHIFTS OF AMINES WITH ^{13}C CHEMICAL SHIFTS IN ANALOGOUSLY CONSTITUTED COMPOUNDS. THE LOWER LINE IS FOR PRIMARY AMINES (RNH_2) AND THE UPPER LINE FOR SECONDARY AMINES (RNR' , WHERE R AND R' MAY BE THE SAME OR DIFFERENT). THE POINT MARKED $\text{C}_6\text{H}_5\text{NH}_2$ IS THAT FOR BENZENAMINE.²³

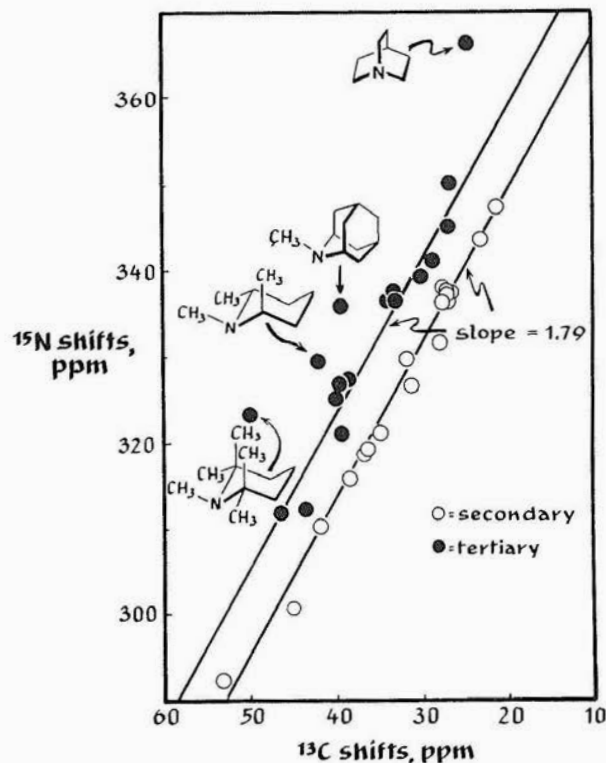
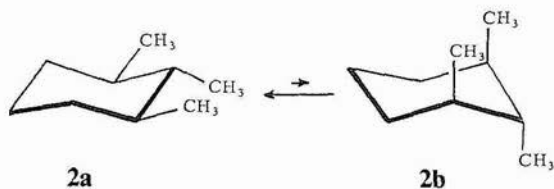


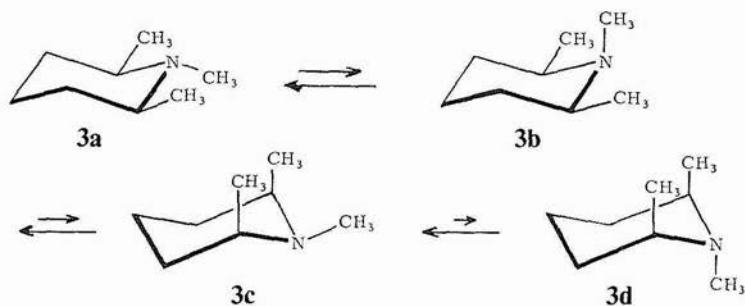
FIG. 7. CORRELATION OF ^{15}N AND ^{13}C SHIFTS FOR VARIOUS SECONDARY AND TERTIARY AZACYCLOHEXANES.²¹ FOR ALL OF THE COMPOUNDS SHOWN, THE NITROGEN IS INCLUDED IN AT LEAST ONE CYCLOHEXANE RING.

azacyclohexanes and aliphatic tertiary amines has clearly shown that tertiary amines give more complex behavior.

In the first place, it will be seen from figure 7 that, even excluding four special cases to be discussed below, the shifts of the tertiary azacyclohexanes show rather more scatter than the secondary azacyclohexanes when one tries to correlate their ^{15}N shifts with the ^{13}C shifts of analogously substituted hydrocarbons.²⁴ There are a number of possible reasons for this, but certainly an important one is the fact that an *N*-substituted azacyclohexane usually has more conformational freedom than the corresponding hydrocarbon. For example, while *trans-trans*-1, 2, 3-trimethylcyclohexane (compound 2) has two possible conformations, with **2a** (three equatorial methyl groups) being expected to be favored over **2b** (three axial methyl

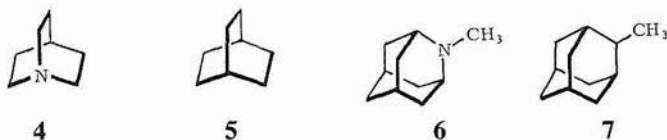


groups), *N*-methyl-*cis*-2, 6-dimethylazacyclohexane (compound 3) has four possible equilibrating conformations. Of these, conformer **3a** is expected to be more favorable, but conformer **3b** is very likely to make up a significant fraction of the equilibrium population and, if this is so, the ^{15}N shift will surely be perturbed toward higher fields, away from the correlation line (as is observed), as compared to the ^{13}C shift of compound 2.

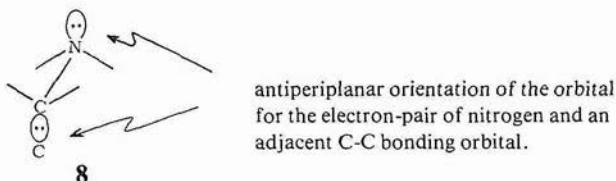


It is unlikely that conformational mobility is the whole story of deviations of the ^{15}N shifts from the correlation line with ^{13}C shifts. The ^{15}N resonance of the conformationally rather rigid 1-azabicyclo[2.2.2]octane (quinuclidine, compound 4) is 15 ppm away from what would be expected from the C1 shift of bicyclo[2.2.2]octane (compound 5), while the ^{15}N shift of the at-least-as-rigid *N*-methyl-2-azabicyclo[3.3.1.1^{3,7}]decane (2-azaadamantane, compound 6) is 13 ppm from predictions based on C2 of 2-

methyltricyclo[3.3.3.1^{1,7}]decane (adamantane, compound 7).



These deviations suggest that a substantial upfield shift is associated with an *antiperiplanar* orientation of the nitrogen electron-pair orbital with respect to adjacent C-C (but not C-H) bonding orbitals, as in partial structure 8.²¹



It will be seen that structure 4 has three such orientations, while structure 5 has two. Two facts support the importance of these orientations. One is that a variety of azacyclohexanes, for example 3(e)- and 9(a)-methyl-1-azabicyclo[4.4.0]decane (3(e)- and 3(a)-methylquinolizidines, compounds 9 and 10), which do not have bond arrangements such as structure 8, have ¹⁵N shifts that correlate with the ¹³C shifts of the corresponding hydrocarbons.

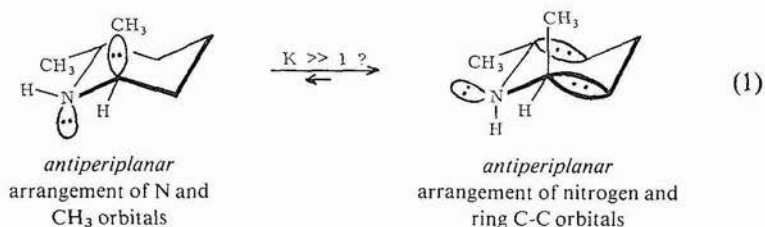


The other fact is that compound 4, compound 6, and *N*-methyl-*trans*-2, 6-dimethylazacyclohexane (compound 11), and *N*-methyl-2, 2, 6, 6-tetramethylazacyclohexane (compound 12) (all with one or more anti-



periplanar arrangements corresponding to structure 8) have much larger hydrogen-bonding and protonation shifts of their nitrogens than do the azacyclohexanes, which do not have favorable conformations for arrangements such as structure 8. This means that, as the electron pair on nitrogen gets tied up in bond formation, the electronic interaction corresponding to 8 gets to be less important, and the anomalous ¹⁵N shifts tend to disappear.

The fact that secondary azacyclohexanes such as *trans*-2, 6-dimethyl- and 2, 2, 6, 6-tetramethylazacyclohexane do not show the *antiperiplanar* effect is the result either of some special influence of the hydrogen bond to nitrogen, or else of the very simple idea that, in these substances, the N-H hydrogen in the equation below is largely axial, a situation that would make the unshared-pair orbital *uniformly antiperiplanar* to the ring C-C orbitals. This possibility seems unlikely from what is known of the axial-equatorial equilibrium of the N-H proton of azacyclohexanes.²⁵



One possible difference in behavior between tertiary and secondary azacyclohexanes is that molecules of the latter can aggregate by hydrogen bonding to each other, $\text{>N-H} \cdots \text{:N} \begin{smallmatrix} \text{H} \\ \diagup \end{smallmatrix}$. This does not suffice to account for the substantial differences in general behavior between these classes of amines, because changes in solvent and concentrations that would be expected to change the hydrogen bonding generally produce effects on the ^{15}N shifts of the secondary azacyclohexanes only on the order of $\pm 1-2$ ppm.

Aliphatic tertiary amines present a very different picture from the tertiary azacyclohexanes,²⁴ as can be seen from figure 8. The scatter of the points from the correlation line is relatively large, and also the slope of 1.39 of the correlation line is very much less than the value of 1.79 for tertiary azacyclohexanes shown in figure 7. The reason for this is not clear, but could well be related to the fact that the rings of azacyclohexanes restrict their tertiary brethren to conformations similar to those of the corresponding hydrocarbons, while aliphatic tertiary amines may not be so constrained.

There are further points of interest about the amine shifts. First, the ^{15}N - ^{13}C correlation lines for the conjugate acids of primary and secondary amines have smaller slopes than those of the primary and secondary amines themselves (see figures 9 and 10). This would appear to indicate that the more closely the amine resembles the hydrocarbon in bond structure, the closer the slopes of the correlation line between ^{13}C and ^{15}N shifts approach unity. This suggests that much of the larger sensitivity of ^{15}N shifts to structural changes when compared with ^{13}C shifts is a property of the unshared pair on nitrogen, and, possibly, that the polarizability of this electron pair is

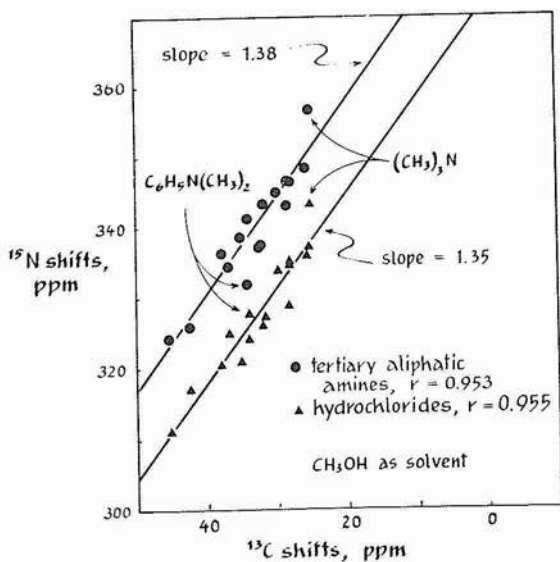


FIG. 8. CORRELATION OF ^{15}N AND ^{13}C shifts of tertiary aliphatic amines and their hydrogen-chloride salts in CH_3OH as solvent.²⁴

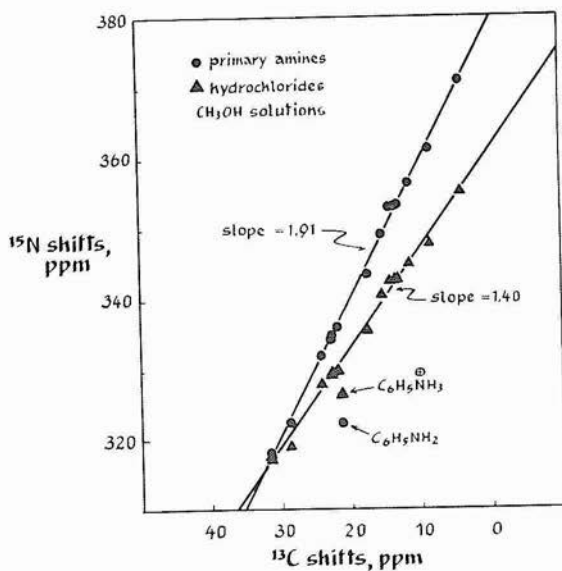


FIG. 9. CORRELATION OF ^{15}N AND ^{13}C SHIFTS of primary aliphatic amines and their hydrogen chloride salts in CH_3OH solutions.²⁴

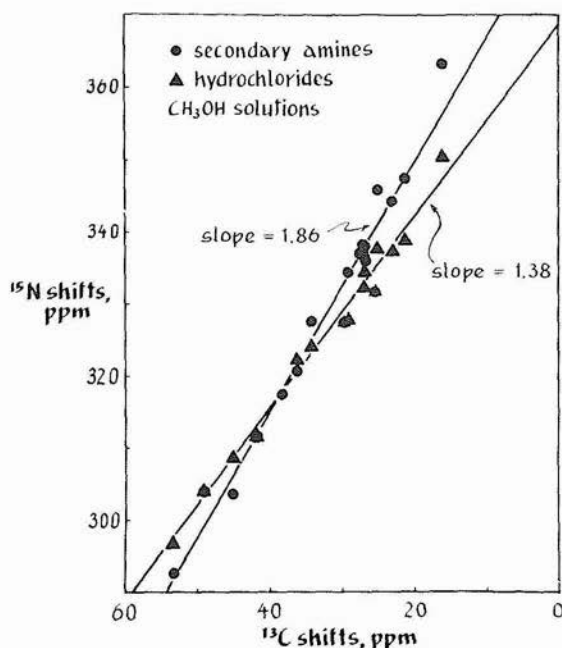


FIG. 10. CORRELATION OF ^{15}N AND ^{13}C SHIFTS of secondary aliphatic amines and their hydrogen-chloride salts in CH_3OH as solvent.²⁴

the important variable. It is interesting in this connection that the slopes of the correlation lines of the amine salts we have studied (including tertiary amines) have slopes within the narrow range of 1.35-1.40, although somewhat different intercepts.²⁴

It will be seen from figures 9 and 10 that the ^{15}N - ^{13}C correlation lines for primary and secondary amines (but not tertiary amines, see figure 8) and their salts cross over. Because the correlations are linear, this leads to the curious fact that the protonation shifts are linear with the ^{15}N shift of the amine itself and, further, if the ^{15}N shift of the amine is far downfield, protonation will result in an *upfield* shift of the nitrogen. The trends are shown in figures 11 and 12, and it turns out that all of the primary and secondary amines in these correlations that give small or *upfield* protonation shifts have branching at the carbons (the α carbons) to which the nitrogens are attached, and the largest *downfield* protonation shifts of the compounds correlated in figures 11 and 12 are CH_3NH_2 and $(\text{CH}_3)_2\text{NH}$ with no branching. It will be seen that the ^{15}N shifts of these amines with branching at the α carbons tend to behave something like that of benzenamine (compound 1), in being downfield of the simpler amines and tending to give small or *upfield* protonation shifts.

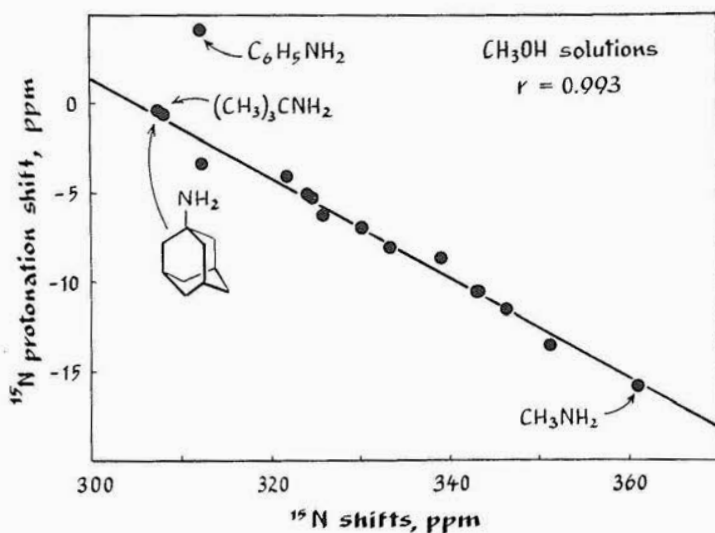


FIG. 11. CORRELATION OF ^{15}N SHIFTS OF primary amines with ^{15}N protonation shifts of the same amines, CH_3OH solutions.²⁴

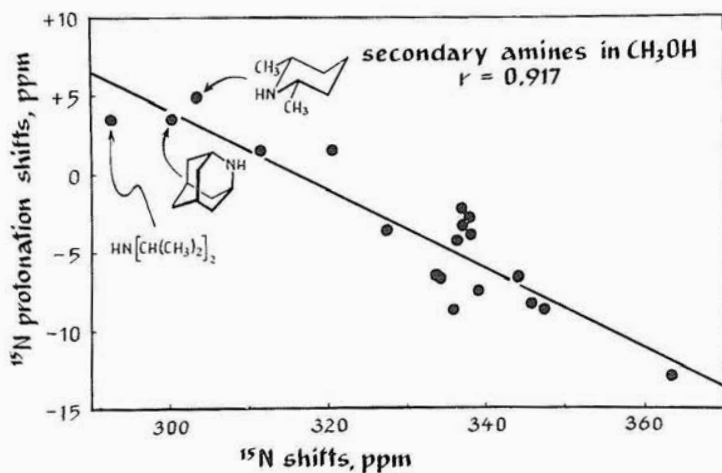
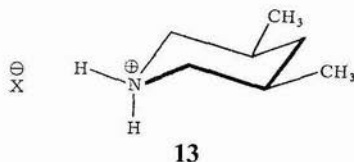


FIG. 12. CORRELATION OF ^{15}N SHIFTS OF secondary amines with ^{15}N protonation shifts of the same amines, CH_3OH solutions.²⁴

The difference in behavior of tertiary amines, for which we postulate important effects arising from the *antiperiplanar* arrangements of groups (4, 6, 11, and 12), is strikingly evident in their protonation shifts, which are large (14 to 24 ppm) but in the *downfield* direction, even though 6, 11, and 12 possess the very branching at the carbons to which the nitrogens are

attached, which is seen in figures 11 and 12 to lead to protonation shifts in the *upfield* direction for primary and secondary amines.²⁴

Influences of solvents on the ^{15}N shifts of primary, secondary, and tertiary amines, as well as those of solvents, concentrations, and counterions on amine salts have been investigated in a systematic way.²⁴ Some large effects have been noted. Thus, for the conjugate acid of *cis*-3, 5-dimethylazacyclohexane (compound **13**), with the counterion $\text{X}^{\ominus} = \text{Cl}^{\ominus}$, there is a downfield shift of 2.2 ppm when the concentration in CHCl_3 is increased from 7.7 to 10.8 mole %.



Change of X^{\ominus} from Cl^{\ominus} to I^{\ominus} in CHCl_3 results in a downfield shift of 3.8 ppm, and from Cl^{\ominus} to BF_4^{\ominus} in an upfield shift of 7.9 ppm, and thus a shift difference from $\text{X} = \text{I}^{\ominus}$ to BF_4^{\ominus} of 11.7 ppm! The influence of X^{\ominus} in CH_3OH as solvent is much smaller, and with $\text{X}^{\ominus} = \text{Cl}^{\ominus}$, I^{\ominus} , and BF_4^{\ominus} , the shift differences are reduced to 2.9 ppm, with I^{\ominus} and BF_4^{\ominus} only 2.3 ppm apart.²⁴ With azoniabenzene (pyridinium) salts, it has been found that the solvent and counterion effects on the *N*-alkyl salts are much smaller than on the *N*-hydrogen salts.²⁶

Another point of interest, and a highly practical one for structural elucidation, is that ^{15}N shifts of azacyclohexanes exhibit the pattern of shift differences where axial and equatorial substituents are involved, a pattern that is such a useful feature of ^{13}C NMR spectra.^{4b, 27} The correlation of figure 7 requires that this be so for nitrogens because many of the substances that correlate well have 3-axial methyl substituents (figure 13).^{21, 28} That similar differences are displayed for an amine group axial or equatorial (upfield by >5 ppm for axial nitrogen) is demonstrated by the *cis*- and *trans*-4-*tert*-butylcyclohexanamines (figure 13) and by data for 2-amino-2-deoxy-D-hexose derivatives.²⁹

For virtually all compounds of which one would have sufficient material to take a natural-abundance ^{15}N spectrum, it would be supposed that the structure would have already been established beyond question. This is not always true for substances where we could determine the precise structure by x ray diffraction for the crystalline state, but which might exist in other forms in solution by operation of acid-base or tautomeric equilibria. One such case is sulfaguanidine, for which structure **14** appears in the usual reference works, despite some earlier physical evidence that structure **15** might in fact be correct. The proton-coupled ^{15}N NMR

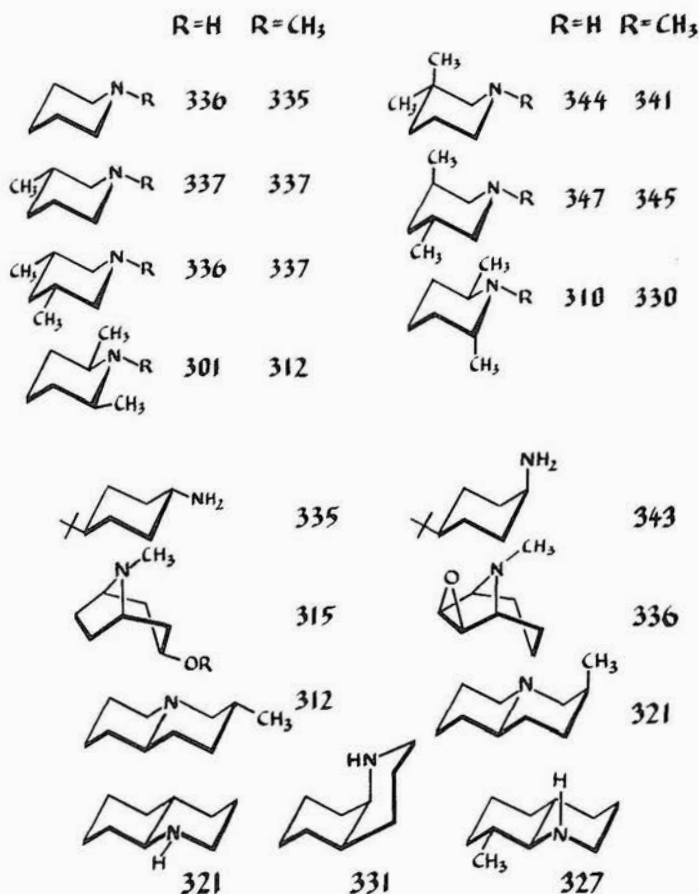


FIG. 13. EFFECT OF AXIAL VS. EQUATORIAL ORIENTATIONS ON ^{15}N shifts of various azacyclohexanes.^{21, 24-28} All shifts are in ppm relative to nitric acid as standard.

spectrum (figure 14) is decisive in distinguishing between **14** and **15**.³⁰ It will be seen that there are two different $-\text{NH}_2$ resonances (1:2:1 triplets) with intensity ratios of 1:2 and a strongly downfield unsplit $-\text{N}=\text{C}$ resonance in agreement with structure **15**. That the $=\text{C}(\text{NH}_2)_2$ nitrogen resonances appear equivalent with the same chemical shift, despite the possibility of *cis-trans* isomerism, may reflect substantial resonance contributions of the valence-bond structure **16** in a nitrogen-inversion transition state.

Another example is provided by the nine-membered lactam, 1-aza-2-cyclononanone. The studies of Huisgen and coworkers³¹ have provided evidence that this substance exists in solution as a mixture of the *cis* and *trans*

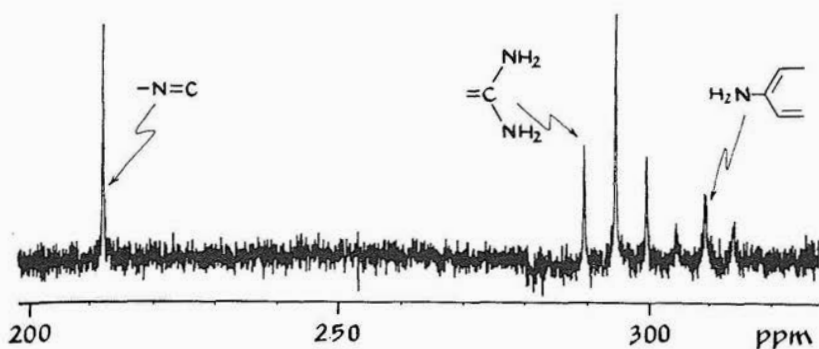
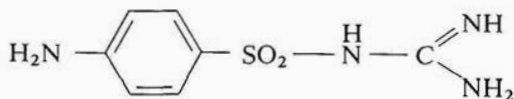
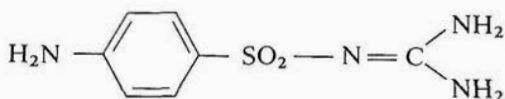
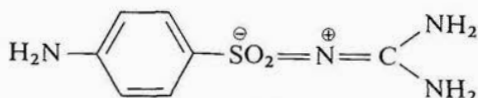
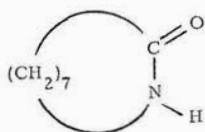
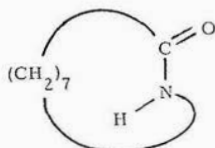


FIG. 14. NATURAL-ABUNDANCE PROTON-COUPLED ^{15}N SHIFTS of sulfaguanidine (structure **15**) in methyldisulfinylmethane solution at 18.23 MHz.¹⁰

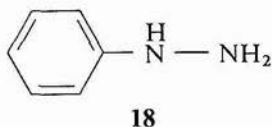
**14****15****16**

isomers **17a** and **17b**, respectively. This despite the fact that the crystalline material is exclusively **17b**.³² However, the ratio of **17a** to **17b** in solution is a matter of some dispute.³³ When the crystals of **17b** are dissolved in trichloromethane at -35° , the ^{13}C spectrum indicated the presence of **17b** in two conformations. When the temperature was raised to $+35^\circ$,

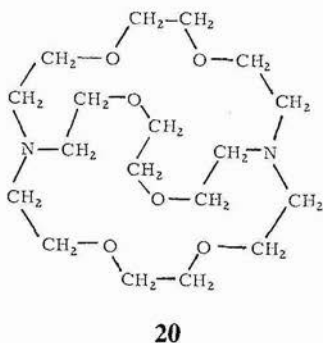
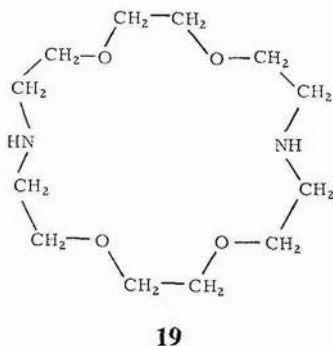
**17a****17b**

isomerization occurred and ^{15}N resonances were observed separated by 3 ppm for **17a** and **17b** in a ratio of 3.5 to 1. In ethanol, the ratio is close to 1:1.³³

The relative rates of N-H hydrogen exchange of the *cis-trans* isomer pair, **17a** and **17b**, in acid or basic solution can be very easily determined from the changes in line shape that occur when exchange becomes rapid on the NMR time scale, because the ^{15}NH couplings are washed out (figure 15).³⁴ This procedure for measuring the N-H exchange rates is very simple and is of special value whenever ^{14}N quadrupole relaxation results in broadening the proton resonance lines to the point where they are not themselves useful in obtaining the exchange rates, as is the case for many amides (most notably the primary ones). The exchange rates of a number of amides of this type have been determined by following changes in line shapes arising from ^{15}NH splitting in water and methylsulfinylmethane solutions.³⁵ The technique has also been applied to intermolecular proton exchanges of phenyldiazane (structure **18**), where, contrary to a published report, $-\text{NH}-$ exchange occurs much more slowly than $-\text{NH}_2$ exchanges (see figure 16).³⁶



A research area of great current interest concerns the interaction between inorganic ions and complexing agents such as the crown ethers and cryptates. We have studied the nitrogen shifts associated with complexation of a number of diamagnetic salts in trichloromethane solution. Some typical results for the *changes* in ^{15}N shift (ppm) on complexation of 1, 10-diaza-4, 7, 13, 16-tetraoxacyclooctadecane (structure **19**), and diaza-4, 7, 13, 16, 21, 24-hexaoxabicyclo[8.8.8]hexacosane (structure **20**) are given in table I (page 168).³⁷



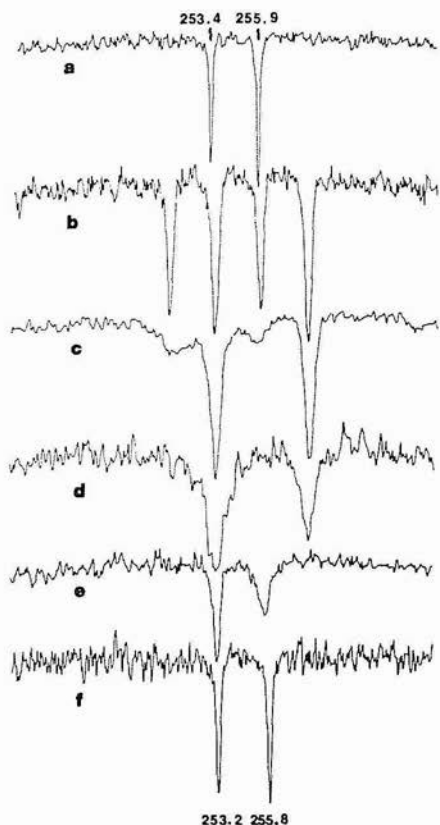


FIG. 15. NATURAL-ABUNDANCE ^{15}N NMR SPECTRA of 2.5 M solution of *cis* and *trans* isomers of 1-aza-2-cyclononanone in methylsulfinylmethane at 25°C; a) with complete ^1H decoupling; b) with gated ^1H noise decoupling (to preserve ^{15}N -H spin-spin couplings); c) as in b) but containing 0.008 M NaOH; d) same as b) but containing 0.02 M NaOH; e) as in b) but containing 0.04 M NaOH; f) as in e) but with complete ^1H decoupling.¹⁴

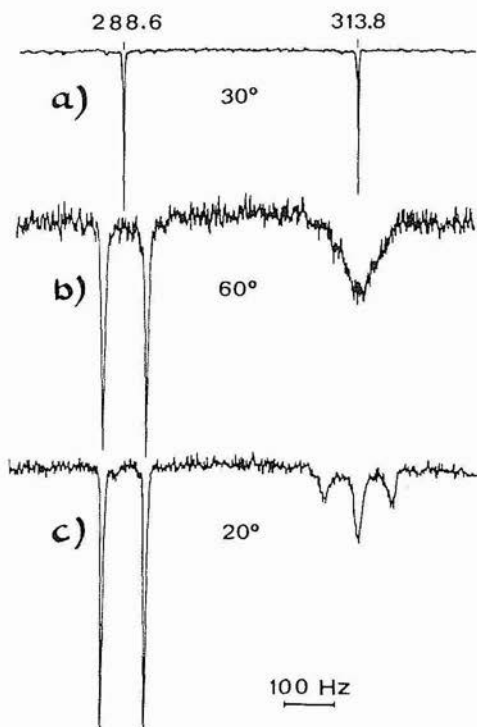


FIG. 16. NATURAL-ABUNDANCE ^{15}N SPECTRA of neat phenyldiazane at 18.23 MHz; a) with ^1H noise decoupling; b) and c) with gated ^1H noise decoupling at two different temperatures.¹⁶

These changes in shift are most commonly paramagnetic (negative sign). Diamagnetic shifts are observed with sodium ion and also lithium ion (using other ring sizes of complexing agents corresponding to **19** and **20**). Silver ion, surprisingly, is diamagnetic with **19** but paramagnetic with **20**. One other trend can be inferred by looking at the difference between the shift changes produced by complexation of **19** and **20** with Na^+ , K^+ , Sr^{2+} , and Ba^{2+} . With these ions, the complexation shift changes for **20** are more dia-

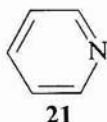
magnetic by about 3 ppm than those for **19**. Silver ion is much different in having a large paramagnetic complexation shift for the change from **19** to **20**. This difference in behavior probably reflects the propensity of silver ion to form more covalent bonds to amine nitrogen than do alkali or alkaline-earth metal ions. The change from diamagnetic to paramagnetic ^{15}N shifts across the sequence, $\text{Na}^+ \rightarrow \text{Ba}^+$, has been previously observed for complexes of ethylenediaminetetracetic acid.³⁸

TABLE I

^{15}N NMR SHIFT CHANGES ON COMPLEXATION OF THE INDICATED IONS WITH STRUCTURES **19** AND **20**

	2H^+	Na^+	K^+	Sr^{2+}	Ba^{2+}	Ag^+	Tl^+
19	-9.8	2.8	-3.2	-4.0	-11.8	5.0	-17.2
20	-8.7	7.1	-0.1	-2.8	-9.6	-4.8	-16.3

The large paramagnetic shift of multiply-bonded nitrogens carrying an unshared pair of electrons, such as $\text{>C}=\ddot{\text{N}}-$ or $-\text{C}\equiv\text{N}:$, and the large upfield shift associated with protonation of the unshared pair were mentioned earlier. This general pattern of shifts has many chemical applications. Of special interest is its use in the study of hydrogen bonding and self association. Hydrogen bonding (and protonation) is known to raise the energy of $n \rightarrow \pi^*$ transitions.³⁹ We then should expect that hydrogen bonding to the unshared pair on $=\ddot{\text{N}}-$ or $\equiv\text{N}:$ should result in an upfield (diamagnetic) shift of the ^{15}N resonance. This effect has long been recognized for azabenzene (pyridine, structure **21**) and related azines^{40,41} and recently has been studied in some detail.²⁶



After correction for bulk-susceptibility effects, it turns out that the ^{15}N resonance of **21** in the gas phase is only about 2 ppm downfield of a dilute solution in cyclohexane. There is a substantial upfield shift of 3-4 ppm with the change from cyclohexane to benzene as solvent, or to neat **21**. These shifts suggest charge-transfer or comparable association of the nitrogen lone pair of one molecule of **21** with an aromatic ring of another molecule,

which is not unreasonable in view of the fact that **21** boils higher than benzene. The ¹⁵N shift of **21** dissolved in methylsulfinylmethane (CH₃SOCH₃), which is a rather polar aprotic solvent and has excellent hydrogen-bond acceptor, but not hydrogen-bond donor, properties, is almost the same as in the neat liquid. This means that the polarity of the solvent *per se* is not a very important characteristic of the solvent in determining the ¹⁵N shifts of **21**—a conclusion that is illustrated in more detail by figure 17, where the ¹⁵N shifts of **21** are plotted against the so-called reaction-field parameter, which is a function of ϵ , the dielectric constant of the solvent $(\epsilon - 1)/(2\epsilon + 2.5)$. Hydrogen-bond donor solvents cause consistent upfield shifts. Thus, going from cyclohexane to CHCl₃ to CH₃OH to H₂O to CF₃CH₂OH gives successive increments of 9.4, 10.6, 2.5, and 10.4 ppm to the ¹⁵N shift, respectively—a total shift of 32.9 ppm! Correlation of these shifts with solvent changes on the $n \rightarrow \pi^*$ energies in the optical spectrum of **21** is not easy, because the $n \rightarrow \pi^*$ transition is only visible as a shoulder on the more intense $\pi \rightarrow \pi^*$ absorption, except in nonpolar solvents. However, the work of Kosower and coworkers⁴² has shown that solvent-dependent energies (*Z*-values) of the charge-transfer excitation of *N*-ethyl-4-methoxycarbonyl-1-azoniabenzene iodide (structure **22**), gives an excellent linear correlation with the solvent dependence of the $n \rightarrow \pi^*$ excitation energy of cyclohexanone and other solvent-dependent ultraviolet

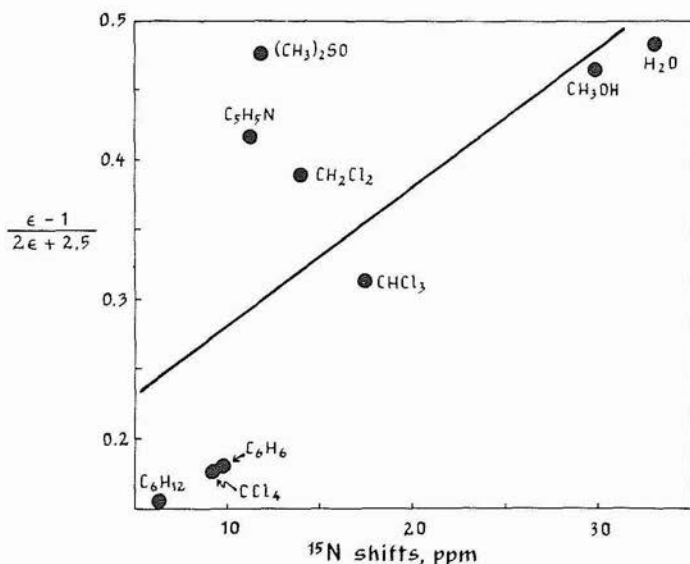
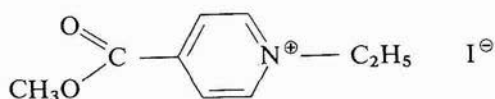


FIG. 17. ATTEMPTED CORRELATION OF ¹⁵N SHIFTS of azabenzene in different solvents with a reaction-field parameter that depends on the dielectric constant ϵ of the solvent.²⁶

**22**

absorptions. Figure 18 shows that the trend of the ^{15}N shift of **21** as a function of solvent is reasonably linear with Z ($r = 0.944$).

Perhaps surprisingly, the large protonation shifts of **21** are not very sensitive to solvent. Thus, CHCl_3 , CH_3SOCH_3 , CH_3OH , and H_2O produce an average protonation shift of 99 ± 2 ppm. The point is that one might expect that strong hydrogen bonding would use up a sizable fraction of the total possible upfield shift of the ^{15}N resonance on protonation, so that when one adds a strong acid to **21** in a strongly hydrogen-bonding solvent, the resulting upfield shift increment would be less than in a non-strongly hydrogen-bonding solvent. This does not happen, and strongly hydrogen-bonding solvents shift *both* the resonance of **21** and of its salt upfield. As mentioned earlier, it appears that solvent and counterion effects are smaller for the *N*-alkyl salts of **21** than for the *N*-hydrogen salts.²⁶

Alkylideneazanol (oximes, structure **23**) illustrate two important points about the ^{15}N shifts of >C=N: types of nitrogens.⁴³

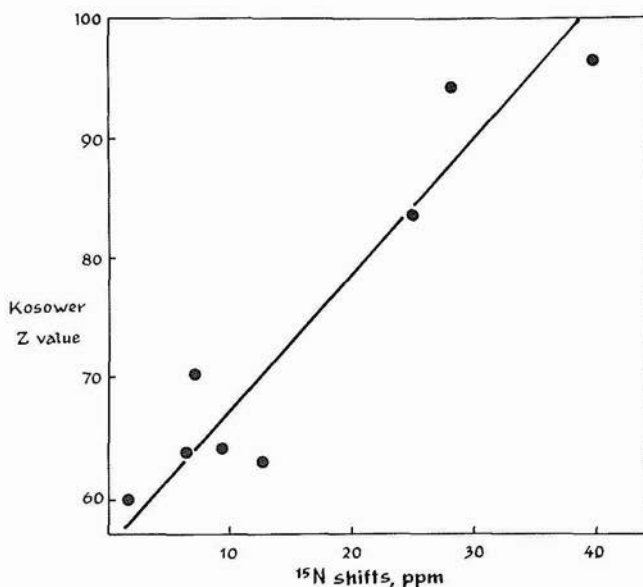
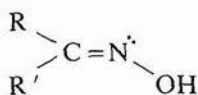
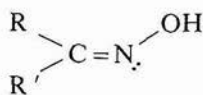
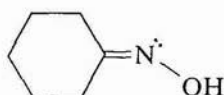
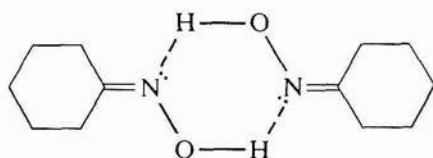
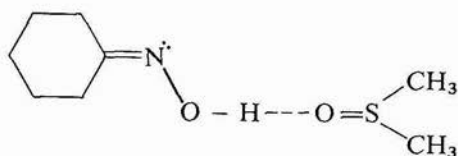


FIG. 18. CORRELATION OF THE ^{15}N SHIFTS of azabenzene in various solvents with the Kosower⁴² Z values of the solvents.²⁶

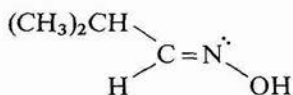
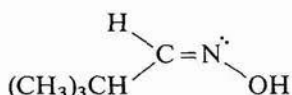
**23a****23b****24**

First, cyclohexylideneazanol (structure **24**) might seem unusual because the resonance of its nitrogen changes less than 2 ppm in the change of solvent from benzene to CHCl_3 to CH_3OH (the corresponding change for azabenzene, structure **21**, is 17.2 ppm). Structure **24** has a fairly strong hydrogen-bonding group in the form of its own OH, however, and can be self-associated as structure **25** or higher aggregates in nonpolar solvents. In CH_3OH , these aggregates may be broken up, with CH_3OH taking over the hydrogen bonding in place of oxime molecules but without much change expected in the ^{15}N shifts. The correctness of this formulation is indicated by two facts. First, when the concentration of **24** is reduced in benzene, the ^{15}N resonance starts to move downfield, as expected for an equilibrium between associated and monomeric oxime. Second, when **24** is dissolved in CH_3SOCH_3 , its ^{15}N resonance is 12.2 ppm *downfield* of its value in benzene. What happens is that aggregates such as **25** are broken down because CH_3SOCH_3 is an excellent hydrogen-bond acceptor and soaks up the hydrogen bonds possible with the OH groups of **24** to give **26**, thus freeing the >C=N: unshared pair from hydrogen bonding and causing a downfield shift.

**25****26**

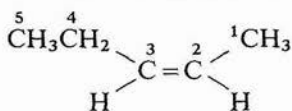
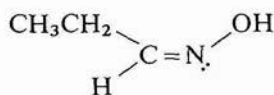
The other point of special interest about the alkylidenazanols are the shifts of the *Z* and *E* (*syn* and *anti*) isomers of the type **23a** and **23b**. When R and R' are different alkyl groups, the difference in nitrogen shifts varies from 2.2 to 5.1 ppm, with larger differences being associated with R and R'

having substantial differences in steric size. Surprisingly, when $R = \text{alkyl}$ and $R' = \text{H}$, the difference in shifts between E and Z is smaller and sometimes negligible. Thus, with $R = (\text{CH}_3)_2\text{CH} -$ (**27a**) and $R' = \text{H}$ (**27b**), the shift difference is only 0.1 ppm as compared to 5.1 ppm for $R = (\text{CH}_3)_2\text{CH} -$ and $R' = \text{CH}_3 -$!

**27a****27b**

Apparently, there is a complicated interplay of steric and electrical effects on the ^{15}N shifts of these compounds.

The relation between the ^{13}C shifts of hydrocarbons and ^{15}N shifts of analogously constituted amines has been discussed earlier *ad nauseam* and, in general, the parallelism seems good, with the ^{15}N shifts being about twice as sensitive to substituent effects as the ^{13}C shifts. The possibility of a similar parallelism for the ^{15}N shifts of alkylidenazanols and ^{13}C shifts of alkenes exists. Thus, would the C2 shift of *cis*-2-pentene (structure **28**) correlate with the ^{15}N shift of *Z*-propylidenazanol (structure **29**)? Clearly

**28****29**

there are differences in that **29** has an OH group where **28** has a CH_3 , but this should not be a serious obstacle, because it has been shown that an $-\text{OH}$ substituted on a hydrocarbon has the same *relative* effect as a CH_3 -group on ^{13}C shifts.⁴⁴ The really important difference between **28** and **29** is the way that the large downfield nitrogen shift of **29** is associated with a paramagnetic effect arising from mixing into the ground state a contribution of an excited state, or states, corresponding to an $n \rightarrow \pi^*$ optical transition. Obviously, because **28** has no n electrons, its ^{13}C shifts cannot be affected in the same way. Figure 19 shows that there is indeed a very good correlation ($r = 0.986$) between the ^{15}N and ^{13}C shifts of pairs of compounds such as **28** and **29** with a slope of 2.18, not much larger than the slopes of amine correlation lines of figures 6-10.⁴⁵

In our present state of knowledge of what determines ^{13}C and ^{15}N shifts, about all, it seems, that can be said about this, is that whatever contribution the $n \rightarrow \pi^*$ excited state makes to the ^{15}N shifts of compounds like **29**, it is not greatly changed by the presence of different substituent groups

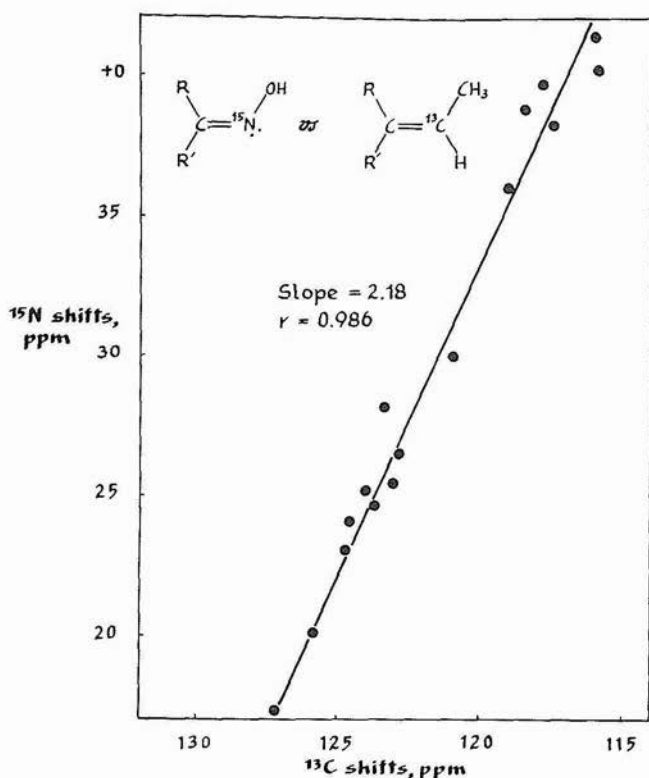
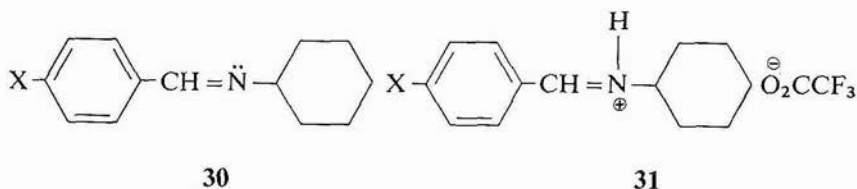


FIG. 19. CORRELATION OF THE ^{15}N SHIFTS of azanols (oximes) with the ^{13}C shifts of methylalkenes.⁴¹

on the carbon of the $\text{C}=\text{N}$ bond. Another indication that this is the case is provided by the 4-substituted phenylmethylenecyclohexanamines (structure **30**) and their conjugate acid salts (structure **31**) with trifluoroethanoic acid. The influence of changes in the X group on the ^{15}N shift of **30** is linear



with the Hammett substituent constant of X, which reflects the ability of X to determine the ionization of X-substituted benzenecarboxylic acids (figure 20).⁴⁶ When the derivatives of **30** are converted to derivatives of **31**, there is a large upfield shift of about 150 ppm, as expected for quenching of con-

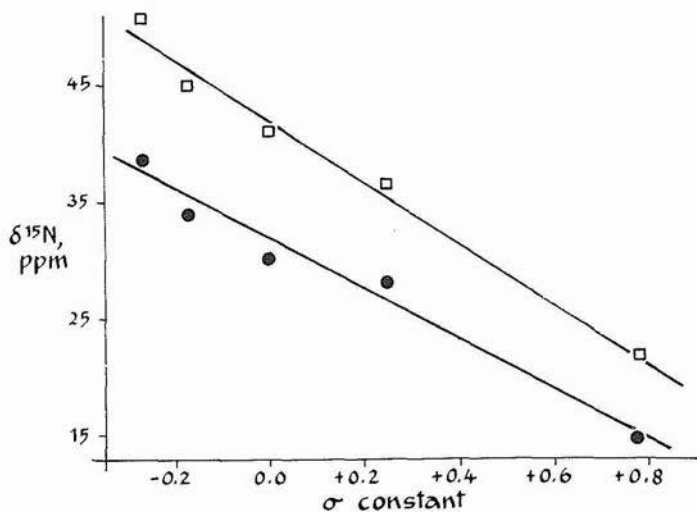


FIG. 20. CORRELATION OF THE ^{15}N SHIFTS of 4-substituted phenylmethylenecyclohexanamines with Hammett substituent constants (σ). Upper line for methanol solutions, lower line for chloroform solutions.⁴⁵

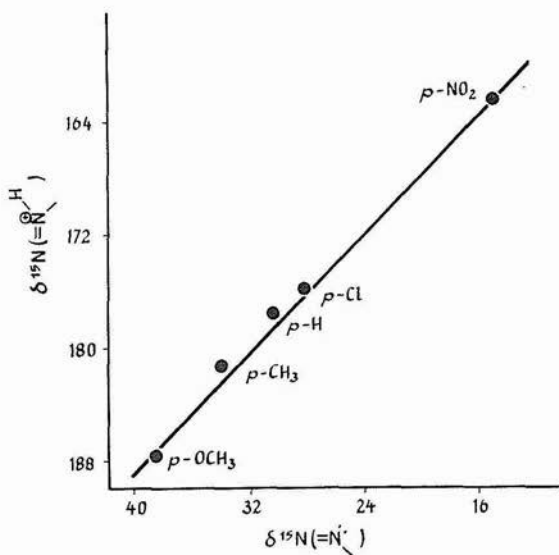
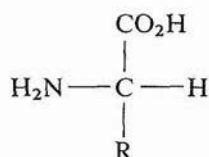


FIG. 21. CORRELATION OF THE ^{15}N SHIFTS of 4-substituted phenylmethylenecyclohexanamines (structure 30) and those of their conjugate acids (structure 31).⁴⁶

tribution of the $n \rightarrow \pi^*$ excited state to the ground state of **30**. The perhaps surprising ancillary result is that the ^{15}N shifts of the derivatives of **30** correlate with those of **31** with a slope of 0.96 and $r = 0.994$ (figure 21). Thus, the effects of the X substituents on the shifts of **30** are virtually the same as for **31**.⁴⁷

Many further applications of ^{15}N NMR to chemistry and biochemistry could be discussed. I will mention here only one possible use of ^{15}N NMR in peptide chemistry, which sheds further light on the importance of the steric effect in influencing ^{15}N chemical shifts. The influence of the saturated R groups of the amino acids alanine, valine, and leucine (parent structure **32**), when combined into di- and tripeptides at the isoelectric point, can be seen from the data given below.^{47, 48}



alanine (Ala), $\text{R} = \text{CH}_3$

valine (Val), $\text{R} =$

leucine (Leu), $\text{R} =$

32

DIPEPTIDES

TRIPEPTIDES



348.9	Gly - Gly	260.4	348.9	Gly - Gly - Gly	260.7
335.0	Ala - Gly	260.4		266.7	
336.8	Leu - Gly	257.1	347.7	Ala - Gly - Gly	260.7
340.2	Val - Gly	255.8		266.6	
			336.4	Leu - Gly - Gly	260.5
348.2	Gly - Ala	245.9		263.7	
335.9	Ala - Ala	246.1	340.1	Val - Gly - Gly	260.5
340.6	Val - Ala	242.6		262.3	
336.7	Leu - Ala	244.0	348.9	Gly - Gly - Ala	246.5
				266.6	
348.9	Gly - Gly	260.4	335.4	Ala - Ala - Ala	247.0
348.4	Gly - Ala	245.9		251.8	
348.6	Gly - Val	252.2	349.6	Gly - Ala - Gly	261.7
348.9	Gly - Leu	248.1		252.2	
			349.1	Gly - Leu - Gly	259.9
335.0	Ala - Gly	260.4		253.8	
335.4	Ala - Ala	246.1			
335.1	Ala - Val	252.2			
335.1	Ala - Leu	248.7			

The nitrogen of each amino-acid residue at the amine (left end) terminus of a peptide is seen to have a characteristic shift that is quite independent of the nature of the other amino acids in the chain. For other than *N*-terminal locations in peptide chains, there are three important shift influences. First, the characteristic shift of the nitrogen of the amino acid itself; second, whether it is at the *C*-terminus; and, third, the nature of the amino acid that is bonded to the nitrogen.

It will be seen from the foregoing that ^{15}N shifts (measured in ppm) are very sensitive to structural changes; indeed, roughly twice as sensitive as ^{13}C shifts, and more than an order of magnitude more sensitive than ^1H shifts. This makes for the possibility of elucidation of quite subtle intra- and intermolecular interactions by ^{15}N NMR, and the purpose of this paper has been to show something of what we know today of the nature and regularity of these interactions. As we come to understand the scope and physical bases involved, we can expect increasing use of ^{15}N NMR to solve critical chemical problems involving nitrogen compounds.

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